510(k) SUMMARY

Cystic Fibrosis Genotyping Assay

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This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and CFR 807.92.

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2. Device Name:

Device Generic Name: Cystic Fibrosis Genotyping Assay
Device Trade Name: Cystic Fibrosis Genotyping Assay
Product Code: NUA
Classification: Class II (21 CFR 866.5900)
510(k) Number: #K062028

3. Equivalence to Legally Marketed Device

The Celera Cystic Fibrosis Genotyping (CF GT) Assay is equivalent to the Tm Bioscience Corporation Tag-It™ Cystic Fibrosis Kit 510(k) #K043011 and to bidirectional DNA sequence analysis (per FDA Special Controls Guidance, CFTR Gene Mutation Detection Systems, issued October 26, 2005).

4. Device Description

The Cystic Fibrosis Genotyping Assay is designed to genotype the normal and mutant alleles at 30 loci of the CFTR gene using purified human genomic DNA. Genotype coverage includes the panel of 23 mutations recommended by the American College of Medical Genetics (ACMG) 2004 guidelines for use in CF population carrier screening.[2] Coverage also includes 9 additional mutations as part of an expanded core panel to support genetic diversity of multiethnic populations that may be underserved by the ACMG panel alone (e.g. Hispanic, African American). In addition to core panel coverage, the assay is designed to detect polythymidine variants (5/7/9T) within intron 8 of the CFTR gene and polymorphisms (I506V, I507V, and F508C) within Exon 10 of the CFTR gene, in accordance with ACMG guidelines.

Purified genomic human DNA is prepared by standard purification methods. A multiplex polymerase chain reaction (PCR) is then performed to amplify the genomic DNA sample with 16 pairs of PCR primers and DNA polymerase. Next, the oligonucleotide ligation assay (OLA) is performed on the CFTR amplicons. Allele-specific OLA probes hybridize to the respective normal, mutant, and variant alleles and become ligated with fluorescent-labeled common probes by the ligase enzyme. The OLA probes are varied in length due to the addition of inert mobility modifiers. The ligated, fluorescent-labeled DNA fragments are separated on the Celera CEGA-16™ Instrument System by electrophoresis. Detection is based on size and fluorescent label. The ligation products are then identified and genotyped by analysis with the CEGA-16 software and assay-specific configuration disk.

The CF GT Assay also contains Reflex OLA reagents for the detection of the polythymidine 5/7/9T variants in intron 8 of the CFTR gene and for the detection of the I506V, I507V, and F508C polymorphisms in Exon 10 of the CFTR gene. The CFTR R117H mutation, along with

the 5T variant of the 5/7/9T polymorphism in intron 8 on the same chromosome (cis), can cause classical CF if another CF mutation is present on the other chromosome. As a result, reflex testing for the 5/7/9T variant with the CF 5/7/9T Reflex OLA assay is recommended when the R117H mutation is detected. The CF Exon 10 Reflex OLA assay is used to verify a homozygous deletion of the F508 or I507 codon and to exclude a potential false-positive result due to interference by certain non-CF causing variants at codons 506, 507, and 508. The CF Exon 10 Reflex OLA assay will distinguish between a true homozygous F508del or I507del from a sample containing one F508del or I507del allele plus the benign variants of I506V, I507V or F508C, respectively. The same software contained on the configuration disk is used to report reflex testing genotyping information.

5. Intended Use

The Cystic Fibrosis Genotyping Assay is a qualitative *in vitro* diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic deoxyribonucleic acid (DNA) isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and Gynecologists (ACOG, 2005) plus additional multiethnic mutations.[1,2,3] The Cystic Fibrosis Genotyping Assay provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening and in confirmatory diagnostic testing of newborns and children. This test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes.

6. Substantial Equivalence / Performance Characteristics

The following table compares the Celera CF GT Kit with the TM Tag-IT CF Kit (#K043011) on basic system characteristics. The clinical trial for the Celera CF GT Kit was performed using sequencing as a gold standard. The performance characteristics the Celera CF GT Kit are well-documented and comparable to the TM Bioscience's product.

Parameter	Celera CF GT Assay	TM Bioscience
Intended Use	The Cystic Fibrosis Genotyping Assay is a qualitative *in vitro* diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic deoxyribonucleic acid (DNA) isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and Gynecologists (ACOG, 2005) plus additional multiethnic mutations. The Cystic Fibrosis Genotyping Assay provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening and in confirmatory diagnostic testing in newborns and children.	The Tag-IT Cystic Fibrosis Kit is a device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the worlds most common and North American-prevalent mutations. The Tag-It Cystic Fibrosis Kit is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.

Parameter	Celera CF GT Assay	TM Bioscience
Contra-indications	This device is not intended for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes.	The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes.
Product Description	The Cystic Fibrosis Genotyping Assay is designed to genotype the normal and mutant alleles at 30 loci of the CFTR gene using purified human genomic DNA. Genotype coverage includes the panel of 23 mutations recommended by the 2004 American College of Medical Genetics (ACMG) guidelines for use in CF population carrier screening. Coverage also includes 9 additional mutations as part of an expanded core panel to support genetic diversity of multiethnic populations. The assay is also designed to detect polythymidine variants (5/7/9T) within Intron 8 of the CFTR gene and polymorphisms (I506V, I507V, and F508C) within Exon 10 of the CFTR gene, in accordance with ACMG guidelines.	The Tag-It™ Cystic Fibrosis Kit tests for 39 mutations and 4 polymorphisms in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. These mutations include those currently recommended for testing by the ACMG/ACOG plus 16 mutations shown to be associated with CF phenotypes in Caucasian Americans, Hispanic Americans and African Americans.

Parameter	Celera CF GT Assay	TM Bioscience
Type of Test	Multiplex PCR followed by DNA sequencing and OLA reflex testing.	Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplexed fluorescing microparticles, detected by flow cytometry.
Specimen Type	Peripheral Human whole blood (EDTA)	Peripheral Human whole blood (EDTA)
Instrument Systems	CEGA-16™ Instrument System	Luminex 100 IS (Integrated System)
Software	Analysis Software: CEGA-16™ Instrument Software with supplied settings and parameters	Tag-IT Data Analysis Software TDAS CF-I
No Template (Negative) Control	CF Sample Diluent (included in Celera CF Genotyping Assay Kit, Set-Up Module)	ddH2O Control (included in Tag-IT CF Kit)
Positive Control	CFTR Wild Type Control (included in Celera CF Genotyping Assay Kit, Set Up Module)	Recommendation is to use genomic DNA controls similar to sample type with △F508 mutation
Reproducibility	100%	>99.99%
Precision	100%	>99.99%
Accuracy	>99.99% Agreement (vs. bi-directional sequencing)	100% Agreement
	delF508 delI507	△F508 △I507

Parameter	Celera CF GT Assay	TM Bioscience
Mutations Detected	G542X	G542X
	G551D	G551D
	W1282X	W1282X
	N1303K	N1303K
	R553X	R553X
	621+1G→T	621+1G→T
	R117H	R117H
	1717-1G→A	1717-1G→A
	A455E	A455E
	R560T	R560T
	R1162X	R1162X
	G85E	G85E
	R334W	R334W
	R347P	R347P
	711+1G→T	711+1G→T
	1898+1G→A	1898+1G→A
	2184delA	2184delA
	1078delT	1078delT
	3849+10kbC→T	3849+10kbC→T
	2789+5G→A	2789+5G→A
	3659delC	3659delC
	3120+1G→A	I148T
	394delTT	3120+1G→A
	S549N	394delTT
	S549R	Y122X
	V520F	R347H

Parameter	Celera CF GT Assay	TM Bioscience
	3876delA	V520F
	2183AA→G	A559T
	R347H	S549N
	3905insT	S549R
	5/7/9/T	2307insA
	I506V	Y1092X
	I507V	M1101K
	F508C	S1255X
		3876delA
		3905insT
		5/7/9T
		F508C
		I507V
		1898+5G→T
		2183AA→G
		I506V

Stability

The expiration date for the Celera CF GT Assay will be based on real-time stability testing.

Analytical Specificity / Interfering Substances

Commonly occurring biological substances that are present in patient blood were tested for their potential to interfere with the performance of the CF Genotyping Assay in detecting mutations. Potential interfering substances (hemoglobin, bilirubin, triglycerides, and protein) were added to whole blood prior to sample preparation process for purified genomic DNA.

Results indicate that these four substances at the following concentrations did not interfere with the CF GT Assay:

Hemoglobin	600 to 820 mg/dL
Bilirubin	20 mg/dL
Triglycerides	500 mg/dL
Protein	8 g/dL

Clinical Testing

Overview

Clinical studies were performed to determine the performance characteristics of the **Cystic Fibrosis Genotyping Assay**. All studies were performed using materials, equipment and procedures described within the Cystic Fibrosis Genotyping Assay Operator's Manual. Every assay run included the CFTR Wild Type Control (allelic ladder/positive assay control) and No Template Control (CF Sample Diluent as a negative control), which are provided within the assay kit. Multiple reagent lots, testing sites, and operators were involved in performing the studies. Samples were blinded and randomized prior to use by the testing sites.

Bi-directional dideoxy DNA sequencing was performed on all clinical trial samples by an independent supplier operating under applicable good laboratory practices (GLPs) and current good manufacturing practices (cGMP) according to 21 CFR Parts 58, 211 and 820, respectively. Polymerase Chain Reaction (PCR) amplification was performed for fifteen (15) regions of interest (ROI) within each sample. Amplicons were sequenced to provide a minimum of double strand sequence data (4-fold coverage, 2 reads per strand) or, for regions containing a heterozygote insertion/deletion, 4-fold coverage from a single strand.

Accuracy

The objective of this study was to assess the clinical accuracy of the Cystic Fibrosis Genotyping Assay for the targeted normal and mutant alleles. The samples tested included genomic DNA obtained from frozen whole blood and frozen pellets from commercially available cell lines. Results from bi-directional dideoxy DNA sequencing by an independent testing service were used as the gold standard for purposes of assessing accuracy of the Cystic Fibrosis Genotyping Assay.

Sample Set

The study was conducted using a set of 201 samples where each core mutation was present within a minimum of 4 samples. The sample set was comprised of 163 unique samples representing purified genomic DNA (51), frozen whole blood (98), fresh whole blood (6) and frozen cell line pellets (8). Most of the samples (65%) contained a single, heterozygous core mutation. Full segmentation of the sample set is summarized in the following table:

Sample Segments	Number of Unique Samples
One Core CF Mutation	109
➢ *Homozygotes*	*3 (all delF508)*
➢ *Heterozygotes*	*106*
Two Core CF Mutations	23
Exon 10 Polymorphisms without Core CF Mutation*	6
Normal for all targeted loci	25
	Total = 163

*Included to assess the assay's ability to detect the polymorphism when present.

To achieve the target minimum of 5 samples per mutation (one exception: 394delTT), additional independent aliquots were generated from 38 of the unique samples. Thus, the complete sample set consisted of 201 samples (i.e., 163 unique + 38 duplicates). Ultimately, all samples were blinded and randomized prior to shipment to the study sites. An overview of the mutation, polymorphism and variant distribution within each of the complete sample set is provided in the following Mutation, Polymorphism and Variant Distribution Table:

	Mutations & P/Vs[a]	Number of Unique Samples with Mutation(s)	Subset with a Second Mutation	Subset with Core + P/V	Number of Unique Samples with P/V only[b]	Additional Independent Aliquots from the Unique Sample Set[c]	Total Samples with Mutation (Unique + Additional, Independent Aliquots)	Total Samples with P/V	Total Replicates Tested[d]
1	1078delT	4	—e	N/A	N/A	1	5	N/A	20
2	1717-1G>A	5	2 of 5 (delF508)	N/A	N/A	—	5	N/A	20
3	1898+1G>A	3	—	N/A	N/A	2	5	N/A	20
4	2183AA>G	1	1 of 1 (delF508)	N/A	N/A	4	5	N/A	20
5	2184delA	4	1 of 4 (delF508)	N/A	N/A	1	5	N/A	20
6	2789+5G>A	5	1 of 5 (delF508)	N/A	N/A	—	5	N/A	20
7	3120+1G>A	4	—	N/A	N/A	1	5	N/A	20
8	3659delC	4	1 of 4 (G542X))	N/A	N/A	1	5	N/A	20
9	3849+10kbC>T	5	—	N/A	N/A	—	5	N/A	20
10	3876delA	5	—	N/A	N/A	1	6	N/A	24
11	3905insT	3	—	N/A	N/A	2	5	N/A	20
12	394delTT	3	—	N/A	N/A	1	4	N/A	16
13	621+1G>T	5	1 of 5 (711+1G>T) 1 of 5 (delF508) 1 of 5 (G85E)	N/A	N/A	4	9	N/A	36
14	711+1G>T	4	1 of 4 (621+1G>T)	N/A	N/A	1	5	N/A	20
15	A455E	5	1 of 5 (delF508)	N/A	N/A	—	5	N/A	20
16	delF508	26	2 of 26 (1717-1G>A) 1 of 26 (2183AA>G) 1 of 26 (2184delA) 1 of 26(2789+5G>A) 1 of 26 (621+1G>T) 1 of 26 (R117H+5T) 1 of 26 (A455E) 3 of 26 (G542X) 2 of 26 (G551D) 1 of 26 (N1303K) 2 of 26 (R553X) 1 of 26 (R560T) 1 of 26 (S549N) 1 of 26 (W1282X)	2 (I506V) 1 (I507V)	N/A	7	33	N/A	132
17	del1507	7	—	0	N/A	1	8	N/A	32
18	G542X	5	1 of 5 (3659delC) 3 of 5 (delF508)	N/A	N/A	—	5	N/A	20
19	G551D	5	2 of 5 (delF508)	N/A	N/A	—	5	N/A	20
20	G85E	4	1 of 4 (621+1G>T)	N/A	N/A	1	5	N/A	20
21	N1303K	5	1 of 5 (delF508)	N/A	N/A	—	5	N/A	20
22	R1162X	3	—	N/A	N/A	2	5	N/A	20
23	R117H	5	1 of 5 (delF508)	2 (5T/7T) 3 (7T/7T)	N/A	—	5	N/A	20
24	R334W	3	—	N/A	N/A	2	5	N/A	20
25	R347H	4	1 of 4 (R553X)	N/A	N/A	1	5	N/A	20
26	R347P	5	—	N/A	N/A	—	5	N/A	20
27	R553X	4	2 of 4 (delF508) 1 of 4 (R347H)	N/A	N/A	1	5	N/A	20
28	R560T	4	1 of 4 (delF508)	N/A	N/A	1	5	N/A	20
29	S549N	3	1 of 3 (delF508)	N/A	N/A	2	5	N/A	20
30	S549R	2	—	N/A	N/A	3	5	N/A	20
31	V520F	4	—	N/A	N/A	1	5	N/A	20
32	W1282X	6	1 of 6 (delF508)	N/A	N/A	—	6	N/A	24
33a	5T	N/A[f]	N/A	2 (R117H)	0	—	N/A	2	8
33b	7T	N/A	N/A	5 (R117H)	0	—	N/A	5	20
33c	9T	N/A	N/A	0	0	—	N/A	0	0
34	I506V	N/A	N/A	2 (delF508)	1	1	N/A	4	16
35	I507V	N/A	N/A	1 (delF508)	2	3	N/A	6	24
36	F508C	N/A	N/A	0	3	2	N/A	5	20

a. P = Exon 10 polymorphisms (F508C, I506V, and I507V); V = Intron 8 variants (5T, 7T, and 9T)
b. Represents samples that contained an Exon 10 polymorphism without a corresponding core mutation (of either homozygous delF508 or delI507).
c. Represents independent aliquots of individual, unique samples that were used to achieve the target minimum of 4 samples per mutation.
d. Total Replicates Tested = [Total Samples with Mutation] x [2 replicates/sample] x [2 test sites]
e. "—" = zero
f. N/A = not applicable

Accuracy Results

Analysis of results from the core OLA assay indicated 100% agreement with the reference results obtained through sequencing. The following table summarizes the Cystic Fibrosis Genotyping Assay results from the core OLA assay relative to the sequencing results. For each sample, analysis by both methods confirmed that normal alleles were present and detected for all loci excluding the loci that contained the expected mutations.

Target Locus by Mutations	804 Sample Replicate Test Results Per Core OLA Assay (201 Samples/Set x 2 Sets/Site x 2 Sites)		Percent Agreement between Sequencing and Test Results (%)
	Number of Sample Replicates with a Mutation Identified at the Target Locus	Number of Sample Replicates with a Normal/Wild Type Allele Identified at the Target Locus	
1078delT	20	804	100
1717-1G>A	20	804	100
1898+1G>A	20	804	100
2183AA>G	20	804	100
2184delA	20	804	100
2789+5G>A	20	804	100
3120+1G>A	20	804	100
3659delC	20	804	100
3849+10kbC>T	20	804	100
3876delA	24	804	100
3905insT	20	804	100
394delTT	16	804	100
621+1G>T	36	804	100
711+1G>T	20	804	100
A455E	20	804	100
delF508	132	792*	100
delI507	32	804	100
G542X	20	804	100
G551D	20	804	100
G85E	20	804	100
N1303K	20	804	100
R1162X	20	804	100
R117H	20	804	100
R334W	20	804	100
R347H	20	804	100
R347P	20		100
R553X	20	804	100
R560T	20	804	100
S549N	20	804	100
S549R	20		100
V520F	20	804	100
W1282X	24	804	100

*Three samples that contained the delF508 mutation were homozygous mutants. Since these three samples did not contain any normal/wild type alleles at the F508 locus, the number of sample replicates with a wild type allele is lower [804 – (3 samples/set x 2 sets/site x 2 sites) = 792].

Analysis of results from the two reflex OLA assays indicated nearly 100% agreement with sequencing, with the exception of one sample replicate that produced a 5T/7T/9T genotype call per the 5/7/9T OLA Reflex Assay. Sequencing indicated that only the 5T and 7T alleles were present. The results from the two reflex OLA assays are summarized in the following table:

P/V[a]	Test Results (Reflex OLA Assays)			Percent Agreement between Sequencing and Test Results (%)
	Number of Replicates with P/V Detected	Number of Replicates that Did Not Meet the Requirements for Reflex Testing[b]	Number of Replicates with Normal/Wild Type Calls	
5T	8	N/A[c]	0	100
7T	0	N/A	16	100
9T	0	N/A	5[d]	80 (4/5)
F508C	0	20	0[e]	100
I506V	12	4	16[e]	100
I507V	4	20	1[e]	100

a. P = Exon 10 polymorphisms (F508C, I506V, and I507V); V = Intron 8 variants (5T, 7T and 9T)
b. These are counted as correct results since in all cases the Core OLA Assay correctly made a wild type (normal)call in the presence of heterozygous F508C, I506V or I507V polymorphism (as indicated by sequencing).Reflex testing was not performed for these samples per the clinical trial protocol, which included a reflex testing algorithm based on ACMG recommendations
c. N/A = not applicable
d. The 5/7/9T OLA Reflex Assay generated a 5T/7T/9T call for one replicate of sample 6043, which did not match the reference sequencing call (5T/7T).
e. The Exon 10 OLA Reflex Assay is not designed to detect the normal allele F508. In the presence of deletion mutations, the I506 and I507 normal alleles are not seen by the Exon 10 OLA Reflex Assay in most cases; thus, Exon 10 results must always be analyzed with results from the Core OLA Assay.

All 201 samples, classified below into one of four genotypic categories, were tested twice for the presence of 32 mutant alleles, 30 normal alleles, 3 Exon 10 polymorphisms, and 3 polythymidine variants by each of the two independent sites:

25: normal DNA with none of the CFTR mutations within the test panel
140: contained one mutant allele within the core panel
35: contained either two mutant alleles within the core panel or one mutant allele + one polymorphism/variant
1: contained two mutant alleles within the core panel + one variant

Percent agreement between the **Cystic Fibrosis Genotyping Assay** and sequencing is summarized in the following table for all 24,954 genotype calls within the sample set. The results for the Core OLA Assay were compared to sequencing with respect to the mutations and normal alleles that the core assay is designed to identify in accordance with ACMG guidelines.[1,2] When reflex testing was indicated by the Core OLA Assay, the results for the polymorphisms or variants identified by the reflex testing were compared with sequencing. The

Cystic Fibrosis Genotyping Assay uses a testing algorithm based on the ACMG guidelines; therefore, testing for polymorphisms within Exon 10 and variants within the polythymidine tract of Intron 8 is only warranted when specific results are observed in the Core OLA Assay.

Sequencing Result	Test Result (Core + Reflex OLA Assays)			Concordant Call	Total (%)
	Normal / Wild Type Call	Heterozygous Call	Homozygous Call		
Normal / Wild Type	24,145 24,146[a]	0	0	24,145[a]	99.996
Heterozygous	0	796	0	796	100
Homozygous	0	0	12	12	100
Total	24,145 24,146[a]	796	12	24,953	99.996[b]

a. The 5/7/9T Reflex OLA Assay erroneously identified the 9T variant in one replicate of sample 6043.Sequencing confirmed that this sample contained a heterozygous 5T variant and the 7T normal allele, which were also detected by the 5/7/9T Reflex OLA Assay (along with 9T).
b. The one-sided 95% lower confidence limit = 99.98%.

System Failure Rate

A total of 24,954 genotype calls was generated by the **Cystic Fibrosis Genotyping Assay** during the accuracy study, and all but one call matched the sequencing result (failure rate = 0.004%). The single incorrect genotype call was associated with the 5/7/9T Reflex OLA Assay result from a single replicate of sample 6043. The reflex assay erroneously indicated the presence of a 9T variant. One 5T and one 7T variant were also detected; thus, the genotype call was 5T/7T/9T. Sequencing confirmed that the sample contained a heterozygous 5T variant and the 7T normal allele. All other replicates of this sample produced the correct 5T/7T result.

Retest Rate

Of the 804 sample replicates tested, a total of 20 (2.5%) required retest due to a poor reaction or PCR failure, or GeneMapper quality flags that indicated that the sample should be repeated when data results were considered questionable or invalid. For samples that did not initially report a genotype result, the correct result was reported upon repeat testing.

Additionally, injection failures on the genetic analyzer were observed for 53 samples (6.6%). The injections were repeated using the same sample-specific electrophoretic mixes that were prepared for the original injection. Therefore, the genotype call was classified as 'delayed'

since repeating the assay was not required. In some cases, the user chose to limit the reinjection to the individual affected samples. In other cases, all samples within the capillary run or entire plate were reinjected, based on convenience. Reinjections were usually performed immediately after the previous run and were always performed within 48 hours of the addition of Hi-Di™ Formamide to the OLA sample. Upon reinjection, valid results were obtained for each sample. In cases where partial or entire plates were reinjected, the initial, correct genotype calls did not change upon reinjection.

An overview of the entire accuracy study results by mutation is provided in the following Accuracy Study by Mutation Table:

Genotype by DNA Sequencing	Number of Unique Samples and/or Replicates		Number CF GT Calls Before Repeat Testing (Based on Initial Results)					Number of CF GT Calls After Repeat Testing (30 calls per sample x number of replicates)[a]			Total Number of Correct Calls	Percent Agreement with Sequencing (%)
			Correct Calls		Delayed Calls[b] (Re-injections)	Percent Agreement with Sequencing for Initial Results[c]	Repeats[d]	Correct Calls		Missed (In-correct) Calls		
	Site A	Site B	Site A	Site B				Site A	Site B			
3659delC	2	2	60	60	0	100	0	60	60	0	120	100
621+1G>T / delF508	2	2	60	60	0	100	0	60	60	0	120	100
1078delT	10	10	270	210	90	95.0	30	300	300	0	600	100
1717-1G>A	6	6	180	180	0	100	0	180	180	0	360	100
1717-1G>A / delF508	4	4	90	90	60	100	0	120	120	0	240	100
1898+1G>A	10	10	270	300	30	100	0	300	300	0	600	100
2183AA>G / delF508	10	10	270	270	30	95.0	30	300	300	0	600	100
2184delA	6	6	180	150	0	91.7	30	180	180	0	360	100
2184 del A / delF508	4	4	120	120	0	100	0	120	120	0	240	100
2789+5G>A	8	8	240	240	0	100	0	240	240	0	480	100
2789+5G>A / delF508	2	2	60	30	30	100	0	60	60	0	120	100
3120+1G>A	10	10	270	240	60	95.0	30	300	300	0	600	100
3659delC	6	6	180	180	0	100	0	180	180	0	360	100
3849+10kbC>T	10	10	300	270	0	95.0	30	300	300	0	600	100
3876delA	12	12	330	360	30	100	0	360	360	0	720	100
3905insT	10	10	240	300	60	100	0	300	300	0	600	100
394delTT	8	8	240	240	0	100	0	240	240	0	480	100
621+1G>T	8	8	210	240	30	100	0	240	240	0	480	100
621+1G>T / 711+1G>T	4	4	120	120	0	100	0	120	120	0	240	100
711+1G>T	6	6	150	150	30	91.7	30	180	180	0	360	100
A455E	8	8	180	210	60	93.8	30	240	240	0	480	100
A455E / delF508	2	2	60	60	0	100	0	60	60	0	120	100
delF508	2	2	30	60	30	100	0	60	60	0	120	100
delF508 / delF508[e]	6	6	186[e]	96[e]	90	100	0	186[e]	186[e]	0	372[e]	100
delI507	16	16	480	480	0	100	0	480	480	0	960	100
F508C	10	10	270	270	30	95.0	30	300	300	0	600	100

Genotype by DNA Sequencing	Number of Unique Samples and/or Replicates		Number CF GT Calls Before Repeat Testing (Based on Initial Results)					Number of CF GT Calls After Repeat Testing (30 calls per sample x number of replicates)[a]			Total Number of Correct Calls	Percent Agreement with Sequencing (%)
			Correct Calls					Correct Calls				
	Site A	Site B	Site A	Site B	Delayed Calls[b] (Re-injections)	Percent Agreement with Sequencing for Initial Results[c]	Repeats[d]	Site A	Site B	Missed (In-correct) Calls		
G542X	2	2	60	30	30	100	0	60	60	0	120	100
G542X / 3659delC	2	2	60	30	30	100	0	60	60	0	120	100
G542X / delF508	6	6	180	150	30	100	0	180	180	0	360	100
G551D	6	6	180	150	30	100	0	180	180	0	360	100
G551D / delF508	4	4	120	90	30	100	0	120	120	0	240	100
G85E	6	6	120	150	60	91.7	30	180	180	0	360	100
G85E / 621+1G>T	4	4	90	120	30	100	0	120	120	0	240	100
I506V	2	2	30	60	30	100	0	60	60	0	120	100
I506V / delF508[e]	6	6	156[e]	155[e]	30	91.7	31[e]	186[e]	186[e]	0	372[e]	100
I507V	10	10	300	300	0	100	0	300	300	0	600	100
I507V / delF508[e]	2	2	32[e]	62[e]	30	100	0	62[e]	62[e]	0	124[e]	100
N1303K	8	8	240	240	0	100	0	240	240	0	480	100
N1303K / delF508	2	2	60	30	30	100	0	60	60	0	120	100
R1162X	10	10	300	270	0	95.0	30	300	300	0	600	100
R117H / 7T[e]	6	6	126[e]	125[e]	90	91.7	31[e]	186[e]	186[e]	0	372[e]	100
R117H / 5T / 7T[e]	2	2	64[e]	63[e,f]	0	99.2	0	64[e]	63[e]	1	127[e]	99.2
R117H / 5T / 9T / delF508[e]	2	2	64[e]	64[e]	0	100	0	64[e]	64[e]	0	128[e]	100
R334W	10	10	300	300	0	100	0	300	300	0	600	100
R347H	8	8	180	210	60	93.8	30	240	240	0	480	100
R347H / R553X	2	2	60	30	30	100	0	60	60	0	120	100
R347P	10	10	240	300	60	100	0	300	300	0	600	100
R553X	4	4	120	120	0	100	0	120	120	0	240	100
R553X / delF508	4	4	90	60	90	100	0	120	120	0	240	100
R560T	6	6	150	180	30	100	0	180	180	0	360	100
R560T / delF508	4	4	90	120	30	100	0	120	120	0	240	100
S549N	8	8	240	240	0	100	0	240	240	0	480	100
S549N / delF508	2	2	60	30	0	75.0	30	60	60	0	120	100
S549R	10	10	270	270	30	95.0	30	300	300	0	600	100
V520F	10	10	300	300	0	100	0	300	300	0	600	100
W1282X	10	10	300	270	30	100	0	300	300	0	600	100
W1282X / delF508	2	2	60	30	30	100	0	60	60	0	120	100
Wild Type	50	50	1,440	1,290	90	94.0	180	1,500	1,500	0	3,000	100
Overall Testing	402	402	11,128	10,825	1,590	97.9	632	12,088	12,087	1	24,175	99.996
			23,543							24,176		

a. Two lots were used in this study.

b. Reinjection of same OLA reaction used for the first injection. Represents cases where users chose to reinject individual samples as well as entire or partial plates (usually based on convenience). In cases where partial or entire plates were reinjected, the initial, correct genotype calls did not change upon reinjection.

c. Results reflect percent agreement with sequencing prior to repeat testing.

d. Repeat from DNA/PCR. Repeat testing was performed due to a poor reaction, PCR failure, or GeneMapper quality flags indicating questionable or invalid data.

e. Reflex testing included. Exon 10: Additional calls per site (1 call per sample x number of replicates x 2 lots). 5/7/9T: Additional calls per site (1 or 2 variant calls per sample x number of replicates x 2 lots).

f. Initial reflex testing made an erroneous call for the 9T variant for 1 of 4 replicates of sample C6043; correct calls were made for the 5/7T variants in this sample. Operator did not perform follow-up testing for this sample, therefore, it remains a missed call.

Reproducibility

To determine clinical reproducibility for the **Cystic Fibrosis Genotyping Assay**, a single-blinded study was conducted at three external test sites. Each test site had one set of instruments, two operators, and three lots of reagents. Each site also processed the same set of samples. The samples tested included genomic DNA obtained from frozen whole blood and frozen pellets from commercially available cell lines. The expected genotype call for every unique sample was confirmed by DNA sequencing.

Sample Set

The set of 144 reproducibility study samples consisted of three (3) replicates each of forty-eight (48) unique genomic DNA samples derived from frozen whole blood (n = 25), and frozen cell line pellets (n = 23). The frozen whole blood samples contained homozygous normal alleles for each of the targeted loci within the Core OLA Assay, whereas the frozen cell line specimens contained either a single heterozygous mutation, a single homozygous mutation, or compound heterozygous mutations for CF. Each of three test sites received sufficient aliquots of each test panel member to conduct three independent assays (3 assays x 144 samples/assay = 432 results per site). Each assay was performed with a different reagent lot (n = 3) on a different day. Reflex testing was performed as indicated per results of the Core OLA Assay and in accordance with ACMG guidelines. These details are summarized in the following table:

Unique Samples	Replicates per Panel	Independent Core OLA Assays Performed per Test Site	Test Sites	Total Results
25 DNA from Frozen Whole Blood (normal alleles)	3	3	3	1,296 = 432 results per site x 3 test sites
23 DNA from Frozen Cell Lines (mutant alleles)				

Reproducibility Results

Results of the study indicate that the **Cystic Fibrosis Genotyping Assay** has a reproducibility of 100%, with zero (0) missed calls, as shown in the Reproducibility Table below.

Statistical analysis of all 1,296 results within the sample set (48 samples x 27 replicates) indicates 100% agreement between the Core OLA Assay and sequencing, with a one-sided 95% lower confidence limit equal to 99.8%.

Based on results of the Core OLA Assay, two samples required reflex testing. Sample #S009346 had a homozygous delF508 call by the Core OLA Assay. Both sequencing and Exon 10 reflex testing for all replicates (9) confirmed the homozygous delF508 call. The Core OLA Assay result for sample #S009369 contained an R117H mutation, therefore, reflex testing for variants in the polythymidine tract on Intron 8 (i.e., 5/7/9T) was conducted. Specifically, the 5T and 9T variant alleles were identified in this sample. Both sequencing and reflex testing results were in 100% agreement for all calls (18).

Reproducibility Table

	Sample Identification[a]	Disease Causing Mutation(s) Confirmed by Sequencing Call	Number of Sites	Number of Sample Replicates Per Each Site = 3 sample replicates x 3 lots	Number of Assay Calls Per Each Site = 30 calls per sample x 3 replicates x 3 lots	Missed Calls[a]	Percent Agreement with Sequencing[b] (%)
1	S009346	delF508; delF508	3	9	279[c]	0	100
2	S009347	3120+1G>A; 621+1G>T	3	9	270	0	100
3	S009348	delF508; R553X	3	9	270	0	100
4	S009349	G551D	3	9	270	0	100
5	S009350	3659delC; delF508	3	9	270	0	100
6	S009351	dell507	3	9	270	0	100
7	S009352	621+1G>T; 711+1G>T	3	9	270	0	100
8	S009353	621+1G>T; delF508	3	9	270	0	100
9	S009354	621+1G>T; G85E	3	9	270	0	100
10	S009355	A455E; delF508	3	9	270	0	100
11	S009356	delF508; R560T	3	9	270	0	100
12	S009357	N1303K	3	9	270	0	100
13	S009358	G542X; G542X	3	9	270	0	100
14	S009359	W1282X	3	9	270	0	100
15	S009360	2789+5G>A; 2789+5G>A	3	9	270	0	100
16	S009361	3849+10kb C>T; 3849+10kb C>T	3	9	270	0	100
17	S009362	1717-1G>A	3	9	270	0	100
18	S009363	R1162X	3	9	270	0	100
19	S009364	G551D; R347P	3	9	270	0	100
20	S009365	R334W	3	9	270	0	100
21	S009369	R117H; 5T/9T; delF508	3	9	288[d]	0	100
22	S009370	2184delA; delF508	3	9	270	0	100

	Sample Identification	Disease Causing Mutation(s) Confirmed by Sequencing Call	Number of Sites	Number of Sample Replicates Per Each Site = 3 sample replicates x 3 lots	Number of Assay Calls Per Each Site = 30 calls per sample x 3 replicates x 3 lots	Missed Calls[a]	Percent Agreement with Sequencing[b] (%)
23	S009371	1898 +1G>A; delF508	3	9	270	0	100
24	T001	WT (wild type)	3	9	270	0	100
25	T002	WT	3	9	270	0	100
26	T004	WT	3	9	270	0	100
27	T005	WT	3	9	270	0	100
28	T006	WT	3	9	270	0	100
29	T007	WT	3	9	270	0	100
30	T008	WT	3	9	270	0	100
31	T009	WT	3	9	270	0	100
32	T010	WT	3	9	270	0	100
33	T011	WT	3	9	270	0	100
34	T012	WT	3	9	270	0	100
35	T013	WT	3	9	270	0	100
36	T014	WT	3	9	270	0	100
37	T015	WT	3	9	270	0	100
38	T016	WT	3	9	270	0	100
39	T0017	WT	3	9	270	0	100
40	T018	WT	3	9	270	0	100
41	T019	WT	3	9	270	0	100
42	T020	WT	3	9	270	0	100
43	T021	WT	3	9	270	0	100
44	T022	WT	3	9	270	0	100
45	T023	WT	3	9	270	0	100
46	T024	WT	3	9	270	0	100
47	T025	WT	3	9	270	0	100
48	T026	WT	3	9	270	0	100

a. Missed Calls are defined as discrepant results relative to the sequencing result.

b. The one-sided 95% lower confidence limit = 99.1% for each individual sample.

c. An additional 9 calls per site (1 call per sample x 3 replicates x 3 lots) were generated as a result of Exon 10 reflex testing (sample ID S009346).

d. An additional 18 calls per site (2 calls per sample x 3 replicates x 3 lots) were generated as a result of 5/7/9T reflex testing (sample ID S009369).

Precision

The Core OLA assay detected all 23 mutations tested under the study protocol, as well as normal (wild type) alleles, with a precision of 100%. Precision of the assay was determined by comparing the results between sites (a total of 3 sites), between operators (a total of six operators, 2 per site) and between reagent lots (a total of 3 lots) as described in the following table:

Mutation	Percent Correct Calls Made for Each Mutation per the Test Method (Cystic Fibrosis Genotyping Assay) (%)		
	Site to Site	Operator to Operator	Lot to Lot
	3 Sites, n = 432 results per site	6 Operators (n = 288 results for 3 operators n = 144 results for 3 operators)	3 Lots, n = 432 results per lot
1717–1G>A	100	100	100
1898+1G>A	100	100	100
2184delA	100	100	100
2789+5G>A	100	100	100
3120+1G>A	100	100	100
3659delC	100	100	100
3849+10kbC>T	100	100	100
621+1G>T	100	100	100
711+1G>T	100	100	100
A455E	100	100	100
delI507	100	100	100
delF508	100	100	100
G542X	100	100	100
G551D	100	100	100
G85E	100	100	100
N1303K	100	100	100
R1162X	100	100	100
R117H	100	100	100
R334W	100	100	100
R347P	100	100	100
R553X	100	100	100
R560T	100	100	100
W1282X	100	100	100

Each of the three reagent lots was tested on a different day at each site. Comparisons by site, operator and reagent lot were calculated for each of the 23 mutations tested. There were a total of 1,296 test results for each mutation (48 samples x 27 replicates).

Exon 10 reflex testing detected all polymorphisms as "present" or "not present," and 5/7/9T reflex testing detected all variants as "present" or "not present," with a precision of 100%. These results are limited to two samples and 81 test results for each sample (27 sample

replicates x 3 tests each). The following table summarizes the precision results for the two reflex assays.

Reflex Assay	Percent Correct Calls Made by Exon 10 or 5/7/9T Reflex Testing (%)		
	Site to Site	Operator to Operator	Lot to Lot
	3 Sites, n = 27 results per site	6 Operators (n = 9 results for 3 operators, n = 18 results, for 3 operators)	3 Lots, n = 27 results per lot
5/7/9T	100	100	100
Exon 10	100	100	100

An overview of the entire reproducibility study results by mutation is provided in the following Reproducibility Study by the Mutation Table:

Genotype by DNA Sequencing	Number of Samples Replicates			Number CF GT Calls Before Repeat Testing (Based on Initial Results)						Number of CF GT Calls After Repeat Testing (30 calls per sample x 3 replicates x 3 lots)				Total Number of Correct Calls	Percent Agreement with Sequencing (%)
				Correct Calls			Delayed Calls[a] (Re-injection)	Percent Agreement with Sequencing for Initial Results[b]	Repeats[c]	Correct Calls			Missed (Incorrect) Calls		
	Site A	Site B	Site C	Site A	Site B	Site C				Site A	Site B	Site C			
1717-1G>A	9	9	9	270	270	120	60	88.9	90	270	270	270	0	810	100
1898+1G>A / delF508	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
2184delA / delF508	9	9	9	240	270	120	90	88.9	90	270	270	270	0	810	100
2789+5G>A / 2789+5G>A	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
3120+1G>A / 621+1G>T	9	9	9	240	270	120	90	88.9	90	270	270	270	0	810	100
3659delC / delF508	9	9	9	210	270	120	120	88.9	90	270	270	270	0	810	100
3849+10kbC>T / 3849+10kbC>T	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
621+1G>T / 711+1G>T	9	9	9	240	270	120	90	88.9	90	270	270	270	0	810	100
621+1G>T / delF508	9	9	9	240	270	120	90	88.9	90	270	270	270	0	810	100
621+1G>T / G85E	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
A455E / delF508	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
delF508 / delF508[d]	9	9	9	249[d]	276[d]	159[d]	60[d]	88.9	93[d]	279[d]	279[d]	279[d]	0	837[d]	100
delI507	9	9	9	240	240	120	90	85.2	120	270	270	270	0	810	100
G542X / G542X	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
G551D	9	9	9	240	270	150	60	88.9	90	270	270	270	0	810	100
G551D / R347P	9	9	9	270	270	180	60	96.3	30	270	270	270	0	810	100
N1303K	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
R1162X	9	9	9	210	270	180	60	88.9	90	270	270	270	0	810	100
R117H / 5T / 9T / delF508[d]	9	9	9	258[d]	282[d]	138[d]	90[d]	88.9	96[d]	288[d]	288[d]	288[d]	0	864[d]	100
R334W	9	9	9	270	270	180	60	96.3	30	270	270	270	0	810	100
R553X / delF508	9	9	9	240	270	150	60	88.9	90	270	270	270	0	810	100
R560T / delF508	9	9	9	240	270	180	30	88.9	90	270	270	270	0	810	100
W1282X	9	9	9	240	270	120	90	88.9	90	270	270	270	0	810	100
Wild Type	225	225	225	5,430	6,660	3,420	2,820	90.5	1,920	6,750	6,750	6,750	0	20,250	100
Overall Testing	432	432	432	11,007	12,858	6,957	4,230	89.4	3,909	38,961			0	38,961	100
				35,052											

a. Reinjection of same OLA reaction. Represents cases where users chose to reinject individual samples as well as entire or partial plates (usually based on convenience). In cases where plates were reinjected, initial, correct genotype calls did not change with repeat testing.

b. Results reflect percent agreement with sequencing prior to repeat testing.

c. Repeat from DNA/PCR. Repeat testing was performed due to a poor reaction or PCR failure, or GeneMapper quality flags indicating questionable or invalid data.

d. Reflex testing included. Exon 10: Nine additional calls per site (1 call per sample x 3 replicates x 3 lots). 5/7/9/T: Eighteen additional calls per site (2 calls per sample x 3 replicates x 3 lots).

7. Conclusions

The Cystic Fibrosis Genotyping Assay accurately identifies the normal and mutant alleles at 30 loci of the CFTR gene from purified human genomic DNA.

8. References Cited:

1. Gordy, W.W. *et al.* 2001. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. Genetics in Medicine 3 (2): 149-154.
2. Watson, M. *et al.* 2004. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genetics in Medicine 6 (5): 387-391.
3. Update on carrier screening for cystic fibrosis. ACOG Committee Opinion No. 325. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2005; 106: 1465-8.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Celera Diagnostics
c/o Ms Victoria Mackinnon
Vice President, RA/CA
1401 Harbor Bay Pkwy
Alameda, CA 94502

SEP - 7 2007

Re: k062028
Trade/Device Name: Cystic Fibrosis Genotyping Assay
Regulation Number: 21 CFR 866.5900
Regulation Name: CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection system
Regulatory Class: Class II
Product Code: NUA
Dated: August 6, 2007
Received: August 7, 2007

Dear Ms. Mackinnon:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The

FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (240) 276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Robert L. Becker, Jr., M.D., Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number: K062028

Device Name: Celera Cystic Fibrosis Genotyping Assay

Indications for Use:

The Cystic Fibrosis Genotyping Assay is a qualitative in vitro diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and Gynecologists (ACOG, 2005) plus additional multiethnic mutations. The Cystic Fibrosis Genotyping Assay provides information intended to be used for carrier screening in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.

This test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes.

Prescription Use ___X___ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Maria M Chan

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K062028